**Supplementary Materials and Methods**

*Reagents.* LY294002 and wortmannin were purchased from Calbiochem; MG132 and cycloheximide were from Sigma.

*Plasmids*. pCMV5-HA-AKT1 WT, myr-AKT1, and AKT1 dominant negative (DN) were described previously ([1](#_ENREF_1)). Twist1 S42A, T121V/S123A, AVA, and DDD mutants were generated with pCMV-HA-Twist1 as a template using a Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, CA). A series of Twist1 WT and mutants for was subsequently constructed in pCMV-3myc, pGEX-6P-1, and pMX-Flag-puro vectors at *Eco*RI cloning sites. pCMV HA-Myr-AKT2 was subcloned from pBabe puroL Myr-HA-Akt2 (Addgene, plasmid #9018) using *Bam*HI and *Eco*RI digestion. pCMV-Flag-Foxc2 was subcloned from pBabe-Foxc2 (Addgene, plasmid #15535) using *Eco*RI and *Bam*HI digestion. pCMV-Flag-Snail, pCMV-Flag-Trcp, pCMV-Flag-Trcp ΔF and the E-cadherin-luciferase reporter were described previously ([2](#_ENREF_2)). All constructs were confirmed by enzyme digestion and DNA sequencing. Detailed information is available upon request.

*Antibody generation.* The mouse anti-phospho-Twist S42 antibody was raised against a phosphorylated synthetic peptide (C-RGGRKRRSS(p)RRSAGG-NH2) and the mouse anti-phospho-Twist1 T121/S123 antibody was generated from a phosphorylated synthetic peptide (C-VRERQRT(p)QS(p)LNEAFAAL-NH2). Antibodies were generated as previously described (5).

*Generation stable cells by retroviral infection*. Recombinant retroviruses were produced by cotransfecting GP293 cells with a retroviral expression plasmid and VSV-G plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) ([3](#_ENREF_3)). The culture supernatants containing infectious viruses were harvested at 48 h post-transfection, centrifuged to eliminate cell debris, and then filtered through 0.22 m filters. For transducing retroviral constructs, 70% confluent MDCK cells were incubated with virus-containing media plus polybrene (Chemicon International, Inc., Temecula, CA) for 1 day to generate 70% infected MDCK cells for visualizing GFP expression. Stable clones were subsequently selected and maintained in culture media containing 2 g/ml puromycin.

*Phos-tag gel*. Phos-tag acrylamide AAL-107 was purchased from NARD Institute (Amagasaki, Japan). HEK-293T cells transfected with the indicated constructs were lysed with Radioimmunoprecipitation assay (RIPA) buffer. Whole-cell lysates were treated with or without phosphatase (New England BioLabs) at 30˚C for 1 h. The lysates were then separated by 12% SDS-PAGE using 100 mM phos-tag according to the manufacturer’s instructions. The phos-tag gels were washed with 1 mM EDTA for 10 min before being transferred to polyvinylidene fluorine membranes, which were then subjected to Western blot.

*Identification of phosphorylation sites using mass spectrometry*. HeLa cells were transfected with HA-Twist1 and myr-AKT1, and cell lysates were then subjected to IP using anti-Twist1 antibodies followed by mass spectrometry analysis. After protein gel electrophoresis, the gel was stained with Coomassie brilliant blue dye. The band corresponding to Twist1 phosphorylation was then excised and subjected to trypsin digestion. Samples were subsequently isolated using immobilized metal affinity chromatography, and the enriched phosphopeptides were analyzed using micro-liquid chromatography/tandem mass spectrometry. The data were searched against the National Center for Biotechnology Information protein sequence database using the Mascot search engine (Matrix Science. Inc., Boston, MA).

*Reverse transcriptase polymerase chain reaction (RT-PCR)*. Total RNA was extracted using Trizol reagents (Invitrogen, Carlsbad, CA), and 2 g RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) with random oligo (dT) primers. Total cDNA products were amplified by PCR using the primer pairs listed in the Supplementary table. PCR products were then separated by electrophoresis in a 1.5% agarose gel and visualized by ethidium bromide staining.

*Cell synchronization.* HeLa cells were plated in 100-mm tissue culture dishes in DMEM with 10% fetal bovine serum and 100 U of penicillin-streptomycin at the density of reaching 50% confluence on the next day. Cells were synchronized by double thymidine block method. Briefly, cells with 50% confluence were first blocked with thymidine (2 mM) for 19 h. Cells were then washed with PBS, trypsinized, and subcultured in 60-mm tissue culture dishes in fresh DMEM. After 9 h incubation, cells were treated with thymidine (2 mM) again for another 17 h. Afterward, cells were washed with PBS for 3 times, incubated in fresh culture medium, and harvested at the indicated time points. Cells were then fixed with ice-cold ethanol and stained with propidium iodine (50 μg/ml) and subjected to FACS analysis (Beckman Coulter Gallios Flow Cytometer). Data were analyzed using the Kaluza Analysis Software (Beckman Coulter).

*Luciferase reporter assay.* MCF7 stable cells plated into 12-well culture plates were transfected with E-cadherin-Luc and the control *Renilla* luciferase reporter construct, pRL-TK (Promega) as previously described ([4](#_ENREF_4)). Twenty-four hours following transfection, cells were harvested, and subjected to luciferase assay using the dual luciferase assay kit and the TD20/20 luminometer (Promega, Madison, WI). Following normalization with the *Renilla* luciferase activity (transfection efficiency control), mean luciferase activities and standard deviations were derived from three independent experiments.

*Confocal microscopy*. Immunofluorescence was performed as previously described ([5](#_ENREF_5)). Briefly, MCF7 transfectants were seeded on Lab-Tek chamber slides 1 day before the experiment and stained with the indicated antibodies. Slides were examined using a Zeiss Axiovert 200 inverted microscope equipped with a cooled charge-coupled device camera (Carl Zeiss, Thornwood, NY).

*Chromatin immunoprecipitation (ChIP) assay*. The ChIP assay was performed as described previously ([6](#_ENREF_6)). Briefly, MCF7 stable cell lines were incubated with 1% formaldehyde in cell culture media for 20 min at 37°C. Thereafter, the cross-linked cells were washed twice with ice-cold PBS and lysed in SDS buffer containing 1% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1), and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Nuclear extracts were sonicated with three 15-sec pulses at 4°C. Supernatants were then diluted in 10-fold dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl [pH 8.1], and 16.7 mM NaCl) and pre-cleared with 80 µl salmon sperm DNA-protein A-agarose (Upstate Biotechnology) for 2 h at 4°C. The sheared DNA mixture was subjected to IP with 1 g anti-Twist1 antibody (Sigma, F3165), anti-HA antibody (Roche, 1815015), anti-RNA polymerase II antibody (Upstate, 05-623B) or an equivalent amount of normal mouse immunoglobulin G (Santa Cruz Biotechnology) overnight at 4°C. The protein-DNA complexes were then precipitated by 60 µl of pre-cleared protein A-agarose for 1 h at 4°C. After sequential washes, the protein-DNA complexes were eluted, and the DNA was extracted using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). For PCR, 3 l of a 40 l DNA preparation was used for amplifications. The DNA product was analyzed using primer-specific amplification of the E-cadherin promoter between the M1 and M2 E-boxes ([7](#_ENREF_7)),5’-CTC CAG CTT GGG TGA AAG AG-3’ and 5’-GGG CTT TTA CAC TTG GCT GA-3’. The 93-bp E-cadherin promoter region was visualized on a 1.5% agarose gel before analyzing by qPCR.

*Wound-healing assays*. MCF7 cells with stable expression of Twist1 were seeded in 6-well plates and allowed to adhere for 24 h. Wounds were created in the confluent monolayers by scratching a pipette tip across the entire diameter of the dish. The confluent monolayers were then rinsed with ice-cold PBS to remove cellular debris. The wounded cells were incubated in culture media supplemented with 5% FBS for 48 h. To monitor wound closure, time-lapse microscopy was performed using an Axiovert 200M cell observer microscope with a 10x objective (Carl Zeiss), and images were obtained at 2 h intervals for 48 h using a high-resolution charge-coupled device Axiocam HRm (Zeiss, Le Pecq, France) connected to the microscope. Phase-contrast pictures were analyzed using AxioVision software v. 4.8.

*In vitro cell migration and invasion assays*. Invasion assays were performed using Transwell permeable supports (Corning-Costar, Cambridge, MA) with uncoated porous filters (8-μm pore size) as earlier described ([6](#_ENREF_6)). Briefly, the filters were precoated with Matrigel matrix (BD Biosciences, Bedford, MA) and air-dried for 2 h. The cells were serum-starved overnight before the experiment. Approximately 5,000 MCF7 cells with stable Twist1 expression were placed onto the upper chamber in 0.25 ml serum-free DMEM. The outer compartment was supplemented with 0.9 ml DMEM containing 10% FBS. After incubation for 2 days, cells that had migrated to the lower surface of the filters were fixed in 4% paraformaldehyde, visualized with 1% crystal violet, and counted. Values for cell invasion were expressed as the average number of cells per microscopic field over three fields in triplicate as described previously ([8](#_ENREF_8)).

*Mammosphere assay.*To generate mammospheres, cells were grown in serum-free, growth factor enriched conditions in ultra-low adherent plate as described earlier ([9](#_ENREF_9)). Briefly, 70 ~ 80% confluent cells were trypsinized and washed with PBS. Cells were then suspended into a single cell suspension with complete MammoCultTM Medium (Stem cell Technologies) containing 4 μg/ml Heparin and 0.48 μg/ml Hydrocortisone at the concentration of 1,000 cells/ml. 2 ml of single cell suspension was added to each well of a 6-well ultra-low adherent plate. Suspension cultures were incubated for 5 days. Mammospheres with size of more than 100 μm were counted and imaged.

*Treatment protocol.* Tumor metastasis was induced in 4- to 6- weeks old female Balb/c mice by inoculation of 4T1 breast cancer cells (1x105) via tail vein injection. One day after cell injection, the mice were randomly divided into four groups (n = 6 per group). The treatment groups received 200 mg/kg MK-2206 and/or 200 mg/kg resveratrol daily by oral gavage. The control group received the vehicle buffer alone. Mice were allowed to live up to their natural death. Kaplan-Meier survival curves were plotted and statistically analyzed. Lung metastatic colonies were counted with a stereoscopic microscope.

*Generation of stable cells using pGIPZ dual expression lentivirus.* The lentiviral-based shRNA (pGIPZ plasmids) used to knockdown expression of AKT1 or AKT2 was purchased from the shRNA/ORF Core Facility (UT MD Anderson Cancer Center). To create a pGIPZ-shAKT1/HA-AKT1 dual expression construct to knock down endogenous AKT1 and reconstitute HA-AKT1 (by creating a silent mutant which resist to shAKT1) simultaneously, first, we made a silent mutant of HA-AKT1 which resist to shAKT1 construct was developed by performing a site direct mutagenesis with the following primers: 5'- GTACCTGGCCCCCGAGGT**CT**T**A**GAGGACAATGACTACG-3' (forward) and 5'- CGTAGTCATTGTCCTC**T**A**AG**ACCTCGGGGGCCAGGTAC-3' (reverse). Bold and underlined base represents mutated site for silent mutant. And then silent mutant of HA-AKT1 was cloned into pGIPZ-shAKT1 (Thermo Scientfic) which expressing shRNA for endogenous AKT1. Using these dual expression constructs, we established endogenous AKT1 knock-down and HA-AKT1 expressing cell lines. To generate lentivirus-expressing shRNA for AKT1 and HA-AKT1, we transfected 293T cells with pGIPZ-non-silence (for vector control virus) or pGIPZ-shAKT1/HA-AKT1 with FuGENE 6 transfection reagent. Twenty-four hours after transfection the medium was changed, then the medium was collected at 24-hour intervals. The collected medium containing lentiviruses were centrifuged to eliminate cell debris, and filtered through 0.45-µm filters. Cells were seeded at 50% confluence 12 hours before infection, and the media were replaced with medium containing lentivirus. After infection for 24 hours, the medium was replaced with fresh medium and the infected cells were selected with 1 µg/ml puromycin (InvivoGen). After puromycin selection of pGIPZ-shAKT1/HA-AKT1 expressing cells, to generate both AKT1 and AKT2 knockdown and HA-AKT1 expressing cells, we infected with a lentivirus which expressing shRNA of AKT2 and sorted GFP positive cells.

In addition, we also established both AKT1 and AKT2 knockdown and HA-AKT2 expressing cells using pGIPZ-shAKT2/HA-AKT2 dual expression construct and pGIPZ-shAKT1 construct. We made a silent mutant of HA-AKT2 which resist to shAKT2 construct was developed by performing a site direct mutagenesis with the following primers: 5'- CATCATTGCCAAGGATGA**G**GT**G**GC**A**CACACAGTCACCGAGAG-3' (forward) and 5'- CTCTCGGTGACTGTGTG**T**GC**C**AC**C**TCATCCTTGGCAATGATG-3' (reverse).

**Supplementary References**

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